FEBS Letters 583 (2009) 2093–2099

FEBS Letters

journal homepage: www.FEBSLetters.org

B30.2/SPRY domain in tripartite motif-containing 22 is essential for the formation of distinct nuclear bodies

Gayathri Sivaramakrishnan, Yang Sun, Rajamuthiah Rajmohan, Valerie C.L. Lin *

School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551, Singapore

ARTICLE INFO

Article history:
Received 16 February 2009
Revised 23 April 2009
Accepted 18 May 2009
Available online 28 May 2009

Edited by Ulrike Kutay

Keywords:
Tripartite motif-containing 22
B30.2/SplA and ryanodine receptor
Nuclear body
Nuclear localization

ABSTRACT

Tripartite motif-containing 22 (TRIM22) is an important antiviral protein that forms distinct nuclear bodies (NB) in many cell types. This study aims to identify functional domains/residues for TRIM22's nuclear localization and NB formation. Deletion of the really-interesting-new-gene (RING) domain, which is essential for its antiviral property, abolished TRIM22 NB formation. However, mutation of two critical residues Cys15 and Cys18 to alanine in the RING domain, did not affect NB formation notably. Although the deletion of the putative bipartite nuclear localization signal (NLS) abolished TRIM22 localization and NB formation, the B30.2/SplA and ryanodine receptor (SPRY) domain, and residues 491–494 specifically are also essential for nuclear localization and NB formation.

1. Introduction

Tripartite motif-containing 22 (TRIM22) is a member of the TRIM family of proteins that have a conserved domain structure, consisting of a really-interesting-new-gene (RING) domain, followed by one or two B-box domains, a coiled-coil (CC) domain and a B30.2/SplA and ryanodine receptor (SPRY) domain at its C-terminus [1]. The RING domain in several tripartite motif-containing (TRIM) proteins has E3 ligase activity, a function that appears to be critical for the roles of these proteins in growth, development and antiviral activity [2–6]. In several TRIM proteins, B-box domains mediate protein–protein interactions [7,8]. The CC domain is largely responsible for homomultimerization [1,8,9]. On the other hand, the function of the B30.2/SPRY domain is not well defined. Amino acid variability in the hydrophilic regions and the inherent flexibility found in certain regions of the B30.2/SPRY domain suggest that these domains can modulate the conformation of TRIM proteins and thereby determine their specificities and regulate their functions [10–12]. The different domains in TRIM proteins can also work coordinately to modulate TRIM protein localization and elicit specific functions as shown for TRIM5α and the Xenopus TRIM protein Nuclear Factor 7 [1,13,14].

TRIM22 was first identified as an interferon-induced protein and named stimulated transacting factor of 50 kDa. It has also been shown to be p53-inducible and to inhibit clonogenic leukemic cell growth and HIV-1 infection in several cell types when overexpressed [15–17]. There is also evidence that TRIM22 interferes with viral gene transcription and Gag protein localization [18,19]. While the ability of TRIM22 to inhibit HIV-1 replication was shown to depend on RING-finger cysteine residues at positions 15 and 18 (Cys15, Cys18), the E3 ligase activity of TRIM22 and its ability to self-ubiquitinate depended on Cys15 [2,19].

Nuclear bodies (NB) are nuclear subdomains that are implicated in a number of nuclear functions including ribogenesis, regulation of transcription and the cell cycle [20,21]. Cajal bodies are better characterized NB marked by the presence of p80-coilin [21]. Several TRIM family proteins including PML/TRIM19, TIF1/TRIM24 and RFP/TRIM27 also form NB. The best characterized one is the promyelocytic leukemia (PML) body which was first identified as a fusion of PML's N-terminal RBCC domain with the retinoic acid receptor alpha in acute promyelocytic leukemia [22,23]. PML has been found to be involved in DNA repair, apoptosis, and telomere maintenance and antiviral function [20,24–26].

We have recently reported that endogenous TRIM22 forms distinct NB that undergo dynamic changes during cell cycle progression, in which TRIM22 NB started to form in the early G0/G1 phase but became speckle-like in the S-phase and completely dispersed in mitosis [27]. The TRIM22 NB did not co-localize with PML NB but up 35% of TRIM22 NB overlapped or were found adjacent to Cajal bodies. These observations suggest the TRIM22 NB is

Abbreviations: CC, coiled-coil; DAPI, 4′,6-diamidino-2-phenylindole; FCS, fetal calf serum; NB, nuclear bodies; NLS, nuclear localization signal; PML, promyelocytic leukemia; RING, really-interesting-new-gene; SPRY, SplA and ryanodine receptor; TRIM, tripartite motif-containing; TRIM22, tripartite motif-containing 22

* Corresponding author. Fax: +65 6791 3856.
E-mail address: cllin@ntu.edu.sg (V.C.L. Lin).

0014-5793/$36.00
doi:10.1016/j.febslet.2009.05.036

important in regulating cell cycle progression. The aim of this study was to identify the domain/residues that are important in TRIM22 nuclear localization and NB formation. The results revealed that the RING domain, but not two of the functional residues Cys15 and Cys18, is required for TRIM22 NB formation. While the putative bipartite nuclear localization signal (NLS) [18] does contribute to nuclear localization, the B30.2/SPRY domain and residues 491–494 specifically, are critical for nuclear localization and the NB formation. These findings not only provide the basis for further elucidating the structure–function relationship of TRIM22 but also offer insight into the contribution of different domains to NB formation in general.

2. Materials and methods

2.1. Cell lines and culture

MCF7 cells were from American Type Culture Collection. HeLa cells were from Dr. Koh Cheng Gee (Nanyang Technological University, Singapore). All cells were routinely maintained in phenol-red containing DMEM supplemented with 7.5% fetal calf serum (FCS), 2 mM glutamine and 40 μg/ml gentamycin.

2.2. Cloning of TRIM22 and mutants

The TRIM22 coding sequence was amplified from RZPD clone TRIM22-pCMV-SPORT6 (IMAGE id: 5583800) using Pfu polymerase and verified by sequencing. The TRIM22 amplicon and mutants were cloned into the pXJ-FLAG vector [28] using restriction enzymes from New England Biolabs. TRIM22 Cys15 and Cys18 mutants were generated from FLAG-TRIM22 by site-directed mutagenesis (Stratagene). Point mutations at the C-terminal end were introduced into FLAG-TRIM22 using mutant primers. Inserts and flanking regions were sequenced to ensure tags were in frame.

2.3. Immunofluorescence

Cells were transfected with constructs using Fugene 6 (Roche Diagnostics) for 24–48 h before they were fixed with 3.7% formaldehyde, permeabilized with 0.2% Triton-X 100, and blocked with 2% FCS/PBS for 1–2 h. Cells were incubated with anti-FLAG antibody (M2) (Sigma–Aldrich) in 2% FCS/PBS for 2 h at 37 °C or overnight at 4 °C, and then incubated with Alexa Fluor 633 nm anti-mouse IgG (Invitrogen) and 4′,6-diamidino-2-phenylindole (DAPI) (Sigma–Aldrich) (0.1 μg/ml) in 2% FCS/PBS for 2 h at 37 °C before mounting on glass slides using anti-fade fluorescence mounting media (Invitrogen). Images were acquired as black and white TIFF files with an Olympus Manual Reflected Fluorescence System attached to an Olympus DP30BW Digital Camera (Olympus Singapore Pte. Ltd., Singapore) using a 60X UPlanSApo oil objective. Color was added using DP Manager (Olympus).

2.4. Cell fractionation and Western blotting analysis

MCF7 cells transfected with various expression plasmids for 48 h were pelleted, re-suspended in buffer S1 (10 mM Hepes pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5% NP-40, 10 mM DTT, 5 μg/ml pepstatin A, 5 μg/ml leupeptin, 2 μg/ml aprotinin), incubated on ice for 10 min, before passing through a 21G syringe 10 times. Cytoplasmic fractions were obtained as the supernatant after centrifugation at 1000×*g* for 10 min. The resultant pellet was re-suspended in buffer S2 (10 mM Hepes pH 7.9, 1.5 mM $MgCl_2$, 0.4 M KCl, 0.5% NP-40, 10 mM DTT, 5 μg/ml pepstatin A, 5 μg/ml leupeptin, 2 μg/ml aprotinin), passed through a 29G syringe 10 times, then centrifuged (20 000×*g*, 15 min) to obtain the nuclear fraction in the supernatant. Protein was quantitated and Western blotting analysis was carried out as previously described [29].

3. Results and discussion

To identify regions/residues that are critical for the nuclear localization and NB formation of TRIM22, a series of TRIM22 mutant constructs were cloned into the pXJ-FLAG vector (Fig. 1). Expression constructs expressed proteins of expected sizes (Supplementary Fig. 1). To exclude artifacts resulting from excessive over-expression, images of cells with relatively low levels of fluorescence are presented.

3.1. The RING domain is required for the NB formation but is not necessary for nuclear localization of TRIM22

The RING domain is a zinc-binding motif that typically functions as an E3 ubiquitin ligase to regulate protein stability via the ubiquitination pathway [30]. TRIM22 was found to undergo self-

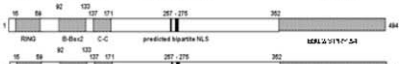
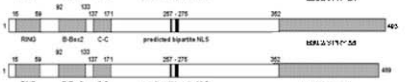
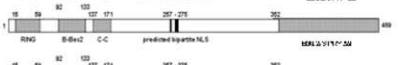
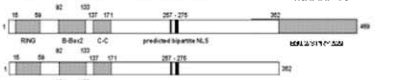
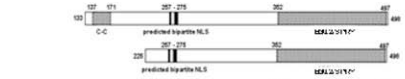
Deletion construct	Protein map	Regions absent	Predicted MW (kDa)	Subcellular localization
TRIM22		--	58.8	N
TRIM22-B30.2/SPRYΔ4		4 aa in B30.2/SPRY	58.3	N
TRIM22-B30.2/SPRYΔ5		5 aa in B30.2/SPRY	58.2	N
TRIM22-B30.2/SPRYΔ9		9 aa in B30.2/SPRY	57.8	N, C
TRIM22-B30.2/SPRYΔ19		19 aa in B30.2/SPRY	56.5	C
TRIM22-B30.2/SPRYΔ29		29 aa in B30.2/SPRY	55.4	C
TRIM22-ΔB30.2/SPRY		B30.2/SPRY	41.5	C
TRIM22-ΔRING		RING	51.1	N
TRIM22-ΔRING/B2		RING, B-box2	43.0	N
TRIM22-ΔRING/B2/CC		RING, B-box2, CC, 53 aa after CC	38.4	N, C
TRIM22-ΔRING/B2/CC/182		RING, B-box2, CC, 182 aa after CC	17.7	C
TRIM22-ΔNLS		Predicted bipartite NLS	57.0	N, C

Fig. 1. TRIM22 deletion mutants were cloned into the pXJ-FLAG vector to investigate the contribution of the different domains to TRIM22 nuclear localization and NB formation.

ubiquitination *in vitro* in a RING domain-dependent manner [2]. The antiviral property of TRIM22 also depends on its RING domain and residues Cys15 and Cys18 are critical for its E3 ligase activity [2,19]. We have shown previously using antibodies specific for TRIM22 that TRIM22 forms distinct NB in several cell lines [27]. FLAG-tagged wild-type TRIM22 localized much like endogenous TRIM22 [27] (Fig. 2A). Deletion of the RING domain abolished TRIM22 NB formation (Fig. 2A). This is consistent with the report that the RING domain is essential for the formation of PML bodies [31]. However, mutating Cys15 and Cys18 to alanine residues (TRIM22-C15A and TRIM22-C15A,C18A) did not abrogate the formation of TRIM22 NB (Fig. 2A). On the other hand, mutations in PML's RING finger domain have been found to alter the subcellular localization of PML from punctuate NB to a diffuse and speckled nuclear staining [22,32] and also to inhibit PML's growth suppressive and apoptotic effects [33,34]. In the case of TRIM22, mutation of Cys15 and Cys18 may not dramatically affect the structure of TRIM22 to the point that NB fail to form, even though these residues have been found to be important for the catalytic activity of the RING domain [2,19]. We speculate that the mutation of Cys15 and Cys18 to alanine did not change the spatial arrangement of the RING domain required for TRIM22 NB formation.

The RING domain does not seem to be required for the nuclear localization of TRIM22 as TRIM22-ΔRING remains nuclear (Fig. 2A and B). Further deletion of the B-box2 domain (TRIM22-ΔRING/B2) did reduce the nuclear localization of the mutant to some extent but the majority of TRIM22-ΔRING/B2 was still localized in the nucleus (Fig. 2B). The protein also appeared to be concentrated at the nuclear periphery (Fig. 2A). This is despite the fact that the 43 kDa TRIM22-ΔRING/B2 may have been able to diffuse through nuclear pores out into the cytoplasm [35]. Unlike TRIM22-ΔRING/B2, TRIM22-ΔRING/B2/CC was localized diffusely throughout the cell by immunostaining. It also appeared that TRIM22-ΔRING/B2/CC was localized more in the cytoplasm by Western blotting analysis

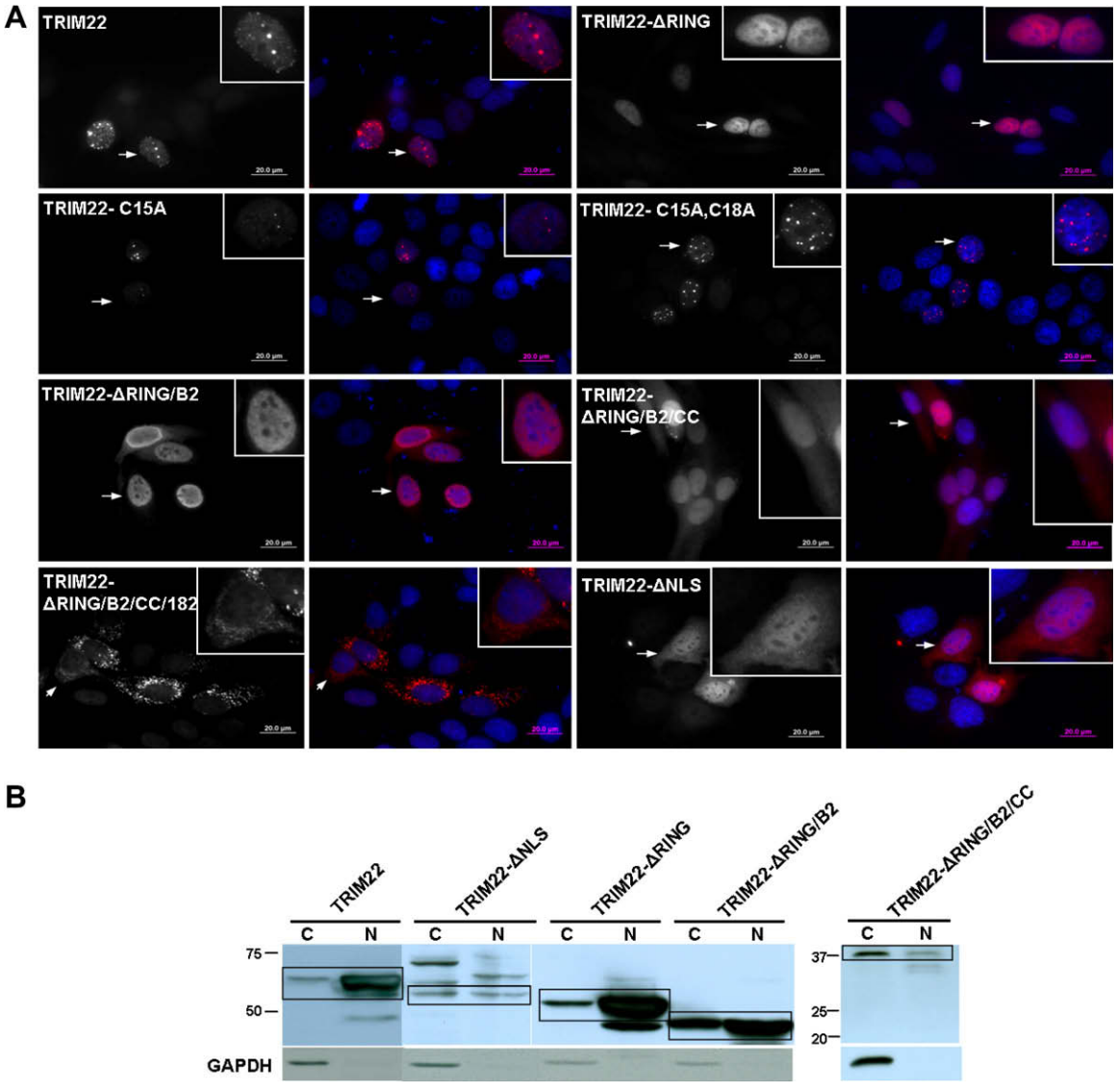


Fig. 2. Subcellular localization of TRIM22 and deletion mutants. (A) MCF7 cells expressing the different deletion mutants were immunostained with anti-FLAG antibody (red) and counterstained with DAPI (blue). Cells were imaged at a 60× magnification. Bars, 20 μm. Images showing staining with the anti-FLAG antibody are presented to the left of the merged image. Insets show representative cells (arrows) enlarged 2×. (B) Cytoplasmic and nuclear levels of TRIM22 and deletion mutants. MCF7 cells transfected with indicated TRIM22 constructs for 48 h were subjected to cell fractionation to obtain cytoplasmic and nuclear fractions. Levels of exogenous proteins in the different fractions were analyzed by Western blotting with anti-FLAG antibody. The cytoplasmic protein GAPDH was probed as a cytoplasmic marker (C: cytoplasmic fraction and N: nuclear fraction).

and this is illustrated in some cells (such as the one pointed out by the arrow) that show more cytoplasmic staining. We speculate that the CC domain may either play a specific role in the nuclear localization of TRIM22, or it mediates homomultimerization of TRIM22-ΔRING/B2 in the nucleus such that the formation of larger complexes prevented the 'leaking' of the protein out of the nucleus.

3.2. The putative bipartite NLS is necessary for the nuclear localization of TRIM22

The bipartite NLS consists of two clusters of positively charged amino acids separated by a linker region of 10–12 amino acids [36]. A putative bipartite NLS in the TRIM22 protein from residues 257–275 (*KR*SESWTL*KKPKSVSKKLK*) has been identified [18] but the function of this sequence as an NLS has not been demonstrated. Deletion of this putative NLS greatly impaired nuclear localization and there seemed to be similar amount of TRIM22-ΔNLS in the cytoplasm and in the nucleus in the Western blotting analysis (Fig. 2B). However, the 57 kDa TRIM22-ΔNLS is unlikely to be freely diffusible so the significant presence of this protein in the cytoplasm is likely caused by the absence of the NLS. We suggest therefore that the predicted NLS is necessary for the efficient import of TRIM22 to the nucleus but may not necessarily be sufficient (Fig. 2). As will be seen later, the B30.2/SPRY domain also contains important signal(s) for nuclear location of TRIM22. The sequence ^{257}*KR*SESWTL*KKPKSVSKKLK*^{275} is likely to be important for protein folding and protein–protein interactions since its deletion completely abolished the formation of TRIM22 NB.

3.3. The B30.2/SPRY domain cooperates with the other regions for nuclear localization and TRIM22 NB formation

The TRIM22 protein contains a 145 aa B30.2/SPRY domain at its C-terminus. The domain has been suggested to play a critical role in the conformations and functions of other TRIM proteins [10–12]. We observed that TRIM22-ΔB30.2/SPRY failed to go into the nucleus and formed cytoplasmic aggregates (Fig. 3A). This was supported by cell fractionation results showing predominant cytoplasmic localization of TRIM22-ΔB30.2/SPRY (Fig. 3B). This suggests that B30.2/SPRY domain is important for nuclear localization of TRIM22. However the C-terminus deletion mutant containing only the B30.2/SPRY domain (TRIM22-ΔRING/B2/CC/182) also localized as cytoplasmic protein. Thus, the B30.2/SPRY domain may collaborate with other sequences such as the putative bipartite NLS to import TRIM22 into the nucleus.

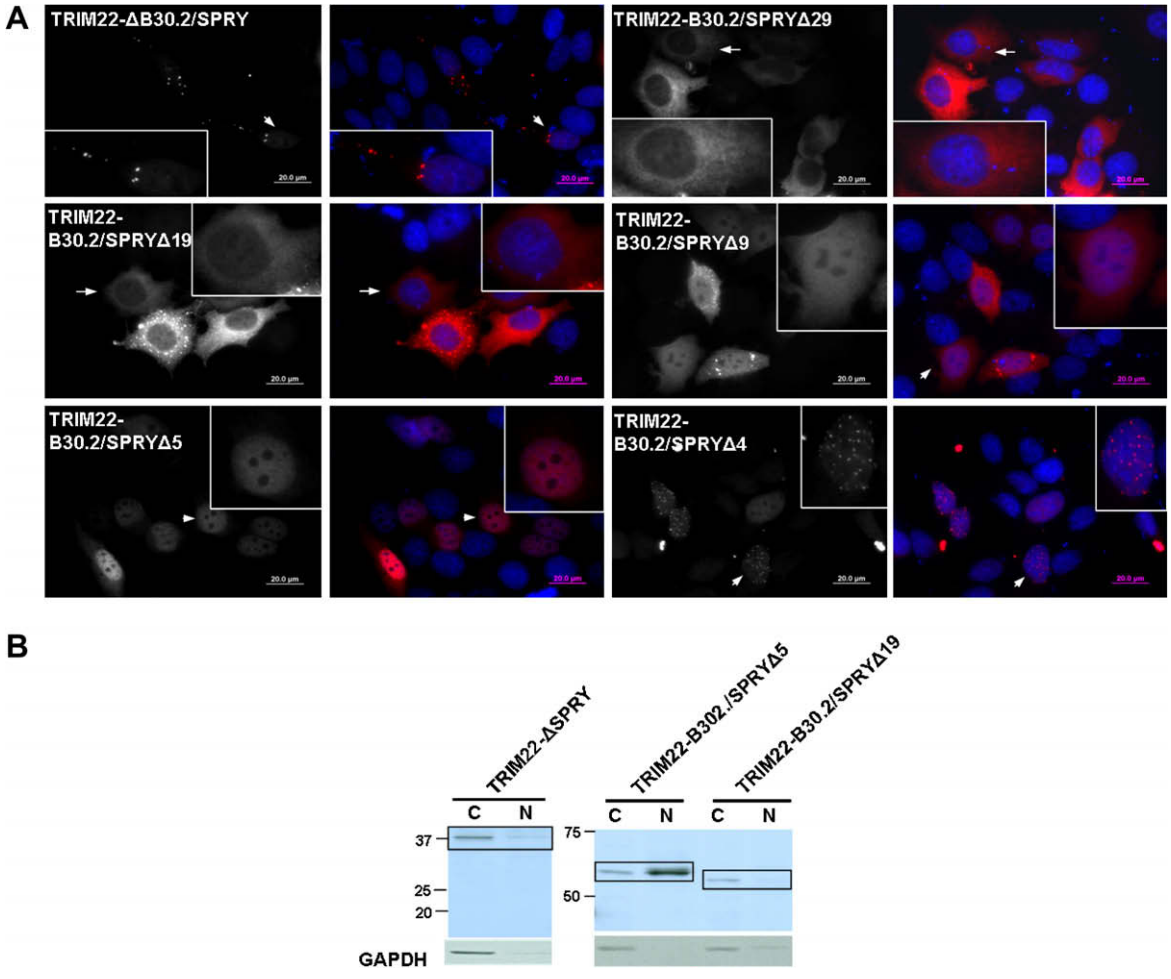


Fig. 3. Localization of TRIM22 mutants lacking 4, 5, 9 and 19 aa of the B30.2/SPRY domain. MCF7 cells expressing the different deletion mutants were immunostained with anti-FLAG antibody (red) and counterstained with DAPI (blue). Cells were imaged at a 60× magnification. Bars, 20 μm. Images showing staining with the anti-FLAG antibody are presented to the left of the merged image. Insets show representative cells (arrows) enlarged 2×. (B) Cytoplasmic and nuclear levels of B30.2/SPRY deletion mutants. MCF7 cells transfected with the indicated constructs for 48 h were subjected to cell fractionation to obtain cytoplasmic and nuclear fractions. Levels of exogenous proteins in the different fractions were analyzed by Western blotting with anti-FLAG antibody. The cytoplasmic protein GAPDH was probed as a cytoplasmic marker (C: cytoplasmic fraction and N: nuclear fraction).

3.4. The last nine amino acids of the B30.2/SPRY domain are critical for the nuclear localization of TRIM22

We went on to identify a minimal stretch within the B30.2/SPRY domain that was important for the nuclear localization of TRIM22. Serial deletions of the protein from the C-terminal end revealed that a mutant lacking the last five aa (TRIM22-B30.2/SPRYΔ5) continued to localize predominantly within the nucleus, whereas the absence of the last nine aa (TRIM22-B30.2/SPRYΔ9) severely compromised nuclear localization (Fig. 3A), a finding which was supported by cell fractionation analysis (Fig. 3B). Therefore, residues 491–494 are important for the nuclear localization of TRIM22. As mutation of these four residues individually did not impair nuclear localization noticeably (data not shown), it is likely that the collective property of these residues, rather than that of the individual residue, is important for the optimal folding and nuclear localization of TRIM22.

3.5. Residues Val493 and Cys494 are required for the formation of distinct TRIM22 bodies

Interestingly, unlike the TRIM22-B30.2/SPRYΔ4 mutant that formed distinct NB much like wild-type TRIM22, the TRIM22-B30.2/SPRYΔ5 mutant was unable to form NB (Fig. 3). Thus, the critical residue for NB formation appears to be Cys494. To investigate if a disulfide bond formed between this cysteine and another cysteine in the protein was critical for NB formation, we mutated Cys494 to either an alanine residue (TRIM22-C494A) or a glutamic acid residue (TRIM22-C494E). Both point mutants continued to form distinct TRIM22 NB (Fig. 4A and B), implying Cys494 is critical for NB formation because of the spatial positioning of the residue rather than the formation of a disulfide bound with another cysteine.

To identify additional determinants of NB formation, we compared the TRIM22 sequence at its C-terminal end with those of its paralogs TRIM6 and TRIM5α which exist as cytoplasmic speckles [1]. Alignment revealed that Val493 in TRIM22 is the only residue within the last nine aa stretch that set it apart from TRIM6 and TRIM5α, all of which have a leucine (L) residue in place of valine (V) (Fig. 4C). Mutating Val493 to a leucine residue (TRIM22-V493L) did not change the localization of the mutant as the NB looked very similar to those formed by wild-type TRIM22 (Fig. 4B). In contrast, mutating Val493 to an alanine residue (TRIM22-V493A) disrupted the formation of regular bodies, resulting in hollow rings in as many as 70% of the structures observed (Fig. 4B). As both valine and leucine are hydrophobic amino acids, they are typically inside of the protein. Conversely, the ambivalent alanine may reside outside of the protein and this position may disrupt partially the amino acid interactions necessary for NB formation.

A few studies have reported the modification of NB into hollow ring-like structures as a result of interaction with other proteins or mutants. For instance, CRAG (a GTPase) induced a ring-like structure of PML NB upon UV irradiation and the PML in this structure

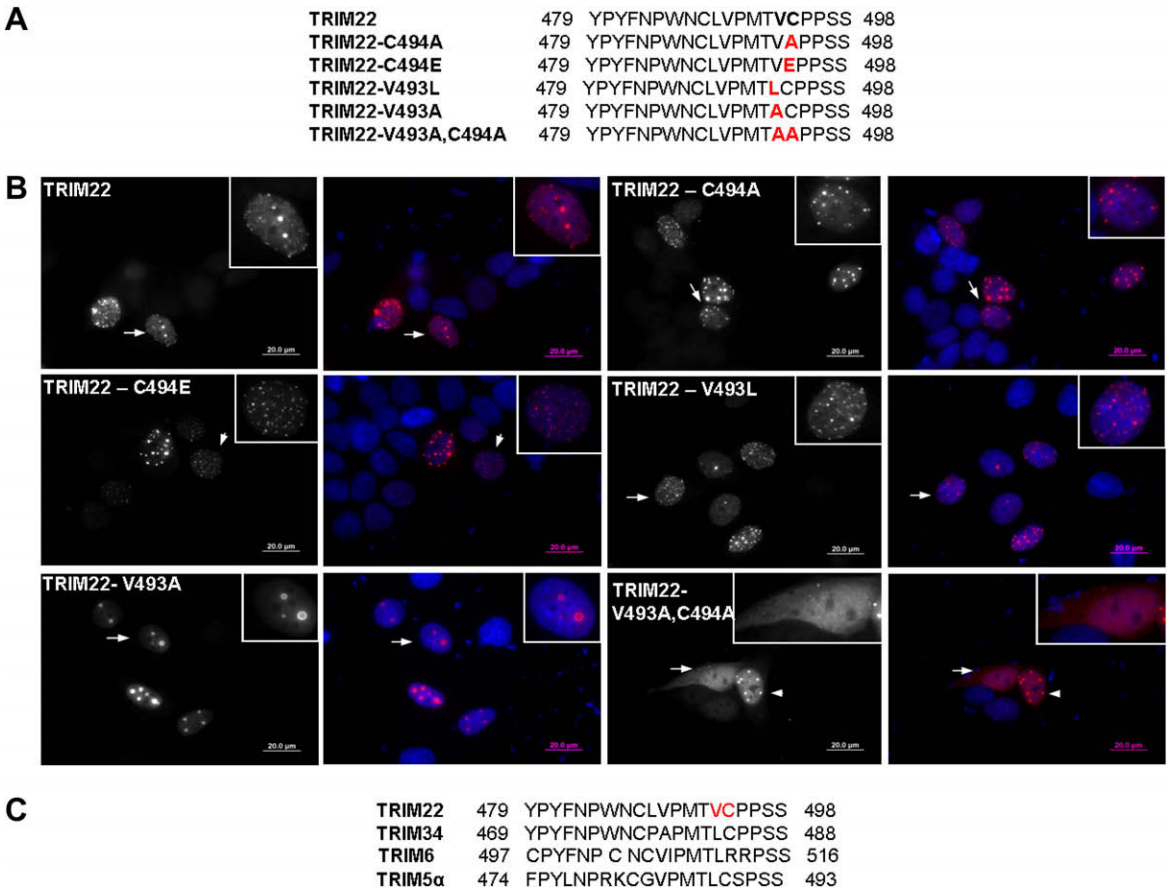


Fig. 4. A hydrophobic residue in position 493 and a residue in position 494 are important for proper nuclear body formation. (A) TRIM22 point mutants (C494A, C494E, V493L, V493A, and V493A,C494A). (B) Cellular localization of TRIM22 point mutants in MCF7 cells. Cells were immunostained with anti-FLAG antibody and counterstained with DAPI. Cells were imaged at a 60× magnification. Bars, 20 μm. Images showing staining with the anti-FLAG antibody are presented to the left of the merged image. Insets show representative cells (arrows) enlarged 2×. The arrowhead in the TRIM22-V493A,C494A panel shows nuclear body formation in a cell expressing larger amount of protein. (C) Alignment of the last 20 residues of human TRIM22 with TRIM34, TRIM6 and TRIM5α.

had ubiquitin ligase activity [37]. The hollow ring structure was also observed when 285 aa was deleted from the C-terminus of p80-coilin [38]. It remains to be tested if the TRIM22 NB and the TRIM22-V493A mutant that formed the hollow ring structure are functionally active.

When both Val493 and Cys494 were mutated to alanine residues (TRIM22-V493A,C494A mutant) the hollow ring structure was no longer present (Fig. 4B). However, the propensity of the TRIM22-V493A,C494A mutant to form regular bodies was greatly reduced as judged by comparing the relative intensity of the NB to the intensity of the nucleoplasmic fluorescence. The double mutant only formed bodies when expressed at high levels where a diffused nucleoplasmic staining was also observed (see cell indicated by the arrowhead). Collectively, our results show that Val493 and Cys494 in the B30.2/SPRY domain of TRIM22 are critical for the presence of TRIM22 within NB. We cannot exclude the possibility that TRIM22 is targeted to pre-existing NB whose component(s) interact with TRIM22 via Val493 and Cys494. Identifying the proteins that interact with TRIM22 within NB would be important for determining if TRIM22 drives the formation of NB, or if other proteins drive this process. It has been reported that mutations in B30.2/SPRY domains of other TRIM proteins such as TRIM5α and TRIM18/MID1 modulate retroviral restriction specificity and cause congenital abnormalities, respectively [39,40]. We suggest that Val493 and Cys494 in TRIM22's B30.2/SPRY domain are likely to be functionally important.

In conclusion, this study has identified domains/residues that are critical for the nuclear localization and NB formation of TRIM22. Firstly, the RING domain is essential for TRIM22 NB formation but mutation of Cys15 and Cys18 did not affect NB formation. It is reasonable to postulate that these two hydrophilic residues are exposed outside for E3 ligase activity but are not required for NB formation. Secondly, the putative bipartite NLS is necessary but not sufficient for the nuclear localization of TRIM22 because the NLS-containing deletion mutant lacking the last nine amino acids was localized mainly to the cytoplasm. Therefore, the B30.2/SPRY domain of TRIM22, in particular residues 491–494, are essential for nuclear localization and NB formation. Finally, we also showed that Val493 and Cys494 are important for the proper formation of TRIM22 NB. This is the first study to demonstrate the critical role of the B30.2/SPRY domain in nuclear localization and NB formation. These findings provide an impetus to elucidating the structure of TRIM22 and its structure–function relationship.

Acknowledgments

The work is supported by grant from the Ministry of Education, the Republic of Singapore to Valerie C.L. Lin.

We wish to thank Dr. E. Manser for pXJ-FLAG plasmid.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2009.05.036.

References

[1] Reymond, A., Meroni, G., Fantozzi, A., Merla, G., Cairo, S., Luzi, L., Riganelli, D., Zanaria, E., Messali, S., Cainarca, S., Guffanti, A., Minucci, S., Pelicci, P.G. and Ballabio, A. (2001) The tripartite motif family identifies cell compartments. EMBO J. 20, 2140–2151.

[2] Duan, Z., Gao, B., Xu, W. and Xiong, S. (2008) Identification of TRIM22 as a RING finger E3 ubiquitin ligase. Biochem. Biophys. Res. Commun. 374, 502–506.

[3] Gack, M.U., Shin, Y.C., Joo, C.H., Urano, T., Liang, C., Sun, L., Takeuchi, O., Akira, S., Chen, Z., Inoue, S. and Jung, J.U. (2007) TRIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity. Nature 446, 916–920.

[4] Trockenbacher, A., Suckow, V., Foerster, J., Winter, J., Krauss, S., Ropers, H.H., Schneider, R. and Schweiger, S. (2001) MID1, mutated in Opitz syndrome, encodes an ubiquitin ligase that targets phosphatase 2A for degradation. Nat. Genet. 29, 287–294.

[5] Urano, T., Saito, T., Tsukui, T., Fujita, M., Hosoi, T., Muramatsu, M., Ouchi, Y. and Inoue, S. (2002) Efp targets 14-3-3 sigma for proteolysis and promotes breast tumour growth. Nature 417, 871–875.

[6] Yamauchi, K., Wada, K., Tanji, K., Tanaka, M. and Kamitani, T. (2008) Ubiquitination of E3 ubiquitin ligase TRIM5alpha and its potential role. FEBS J. 275, 1540–1555.

[7] Borden, K.L., Lally, J.M., Martin, S.R., O'Reilly, N.J., Solomon, E. and Freemont, P.S. (1996) In vivo and in vitro characterization of the B1 and B2 zinc-binding domains from the acute promyelocytic leukemia protooncoprotein PML. Proc. Natl. Acad. Sci. USA 93, 1601–1606.

[8] Cao, T., Borden, K.L., Freemont, P.S. and Etkin, L.D. (1997) Involvement of the rfp tripartite motif in protein–protein interactions and subcellular distribution. J. Cell Sci. 110, 1563–1571.

[9] Cainarca, S., Messali, S., Ballabio, A. and Meroni, G. (1999) Functional characterization of the Opitz syndrome gene product (midin): evidence for homodimerization and association with microtubules throughout the cell cycle. Human Mol. Genet. 8, 1387–1396.

[10] Ohkura, S., Yap, M.W., Sheldon, T. and Stoye, J.P. (2006) All three variable regions of the TRIM5alpha B30.2 domain can contribute to the specificity of retrovirus restriction. J. Virol. 80, 8554–8565.

[11] Rhodes, D.A. and Trowsdale, J. (2007) TRIM21 is a trimeric protein that binds IgG Fc via the B30.2 domain. Mol. Immunol. 44, 2406–2414.

[12] Woo, J.S., Imm, J.H., Min, C.K., Kim, K.J., Cha, S.S. and Oh, B.H. (2006) Structural and functional insights into the B30.2/SPRY domain. EMBO J. 25, 1353–1363.

[13] Beenders, B., Jones, P.L. and Bellini, M. (2007) The tripartite motif of nuclear factor 7 is required for its association with transcriptional units. Mol. Cell Biol. 27, 2615–2624.

[14] Javanbakht, H., Yuan, W., Yeung, D.F., Song, B., Diaz-Griffero, F., Li, Y., Li, X., Stremlau, M. and Sodroski, J. (2006) Characterization of TRIM5alpha trimerization and its contribution to human immunodeficiency virus capsid binding. Virology 353, 234–246.

[15] Izmailova, E., Bertley, F.M., Huang, Q., Makori, N., Miller, C.J., Young, R.A. and Aldovini, A. (2003) HIV-1 Tat reprograms immature dendritic cells to express chemoattractants for activated T cells and macrophages. Nat. Med. 9, 191–197.

[16] Obad, S., Brunnstrom, H., Vallon-Christersson, J., Borg, A., Drott, K. and Gullberg, U. (2004) Staf50 is a novel p53 target gene conferring reduced clonogenic growth of leukemic U-937 cells. Oncogene 23, 4050–4059.

[17] Zhang, J., Das, S.C., Kotalik, C., Pattnaik, A.K. and Zhang, L. (2004) The latent membrane protein 1 of Epstein-Barr virus establishes an antiviral state via induction of interferon-stimulated genes. J. Biol. Chem. 279, 46335–46342.

[18] Tissot, C. and Mechti, N. (1995) Molecular cloning of a new interferon-induced factor that represses human immunodeficiency virus type 1 long terminal repeat expression. J. Biol. Chem. 270, 14891–14898.

[19] Barr, S.D., Smiley, J.R. and Bushman, F.D. (2008) The interferon response inhibits HIV particle production by induction of TRIM22. PLoS Pathog. 4, e1000007.

[20] Bernardi, R. and Pandolfi, P.P. (2007) Structure, dynamics and functions of promyelocytic leukaemia nuclear bodies. Nat. Rev. Mol. Cell Biol. 8, 1006–1016.

[21] Cioce, M. and Lamond, A.I. (2005) Cajal bodies: a long history of discovery. Ann. Rev. Cell Dev. Biol. 21, 105–131.

[22] Kastner, P., Perez, A., Lutz, Y., Rochette-Egly, C., Gaub, M.P., Durand, B., Lanotte, M., Berger, R. and Chambon, P. (1992) Structure, localization and transcriptional properties of two classes of retinoic acid receptor alpha fusion proteins in acute promyelocytic leukemia (APL): structural similarities with a new family of oncoproteins. EMBO J. 11, 629–642.

[23] Rowley, J.D., Golomb, H.M., Vardiman, J., Fukuhara, S., Dougherty, C. and Potter, D. (1977) Further evidence for a non-random chromosomal abnormality in acute promyelocytic leukemia. Int. J. Cancer 20, 869–872.

[24] Mu, Z.M., Chin, K.V., Liu, J.H., Lozano, G. and Chang, K.S. (1994) PML, a growth suppressor disrupted in acute promyelocytic leukemia. Mol. Cell Biol. 14, 6858–6867.

[25] Grobelny, J.V., Godwin, A.K. and Broccoli, D. (2000) ALT-associated PML bodies are present in viable cells and are enriched in cells in the G(2)/M phase of the cell cycle. J. Cell Sci. 113, 4577–4585.

[26] Everett, R.D. and Chelbi-Alix, M.K. (2007) PML and PML nuclear bodies: implications in antiviral defence. Biochimie 89, 819–830.

[27] Sivaramakrishnan, G., Sun, Y., Tan, S.K. and Lin, V.C.L. (2009) Dynamic localization of tripartite motif-containing 22 in nuclear and nucleolar bodies. Exp. Cell Res. 315, 1521–1532.

[28] Manser, E., Huang, H.Y., Loo, T.H., Chen, X.Q., Dong, J.M., Leung, T. and Lim, L. (1997) Expression of constitutively active alpha-PAK reveals effects of the kinase on actin and focal complexes. Mol. Cell Biol. 17, 1129–1143.

[29] Zheng, Z.Y., Bay, B.H., Aw, S.E. and Lin, V.C. (2005) A novel antiestrogenic mechanism in progesterone receptor-transfected breast cancer cells. J. Biol. Chem. 280, 17480–17487.

[30] Lorick, K.L., Jensen, J.P., Fang, S., Ong, A.M., Hatakeyama, S. and Weissman, A.M. (1999) RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. Proc. Natl. Acad. Sci. USA 96, 11364–11369.

[31] Boddy, M.N., Duprez, E., Borden, K.L. and Freemont, P.S. (1997) Surface residue mutations of the PML RING finger domain alter the formation of nuclear matrix-associated PML bodies. J. Cell Sci. 110, 2197–2205.
[32] Borden, K.L., Boddy, M.N., Lally, J., O'Reilly, N.J., Martin, S., Howe, K., Solomon, E. and Freemont, P.S. (1995) The solution structure of the RING finger domain from the acute promyelocytic leukaemia proto-oncoprotein PML. EMBO J. 14, 1532–1541.
[33] Borden, K.L., Campbell-Dwyer, E.J. and Salvato, M.S. (1997) The promyelocytic leukemia protein PML has a pro-apoptotic activity mediated through its RING domain. FEBS Lett. 418, 30–34.
[34] Fagioli, M., Alcalay, M., Tomassoni, L., Ferrucci, P.F., Mencarelli, A., Riganelli, D., Grignani, F., Pozzan, T., Nicoletti, I. and Pelicci, P.G. (1998) Cooperation between the RING + B1-B2 and coiled-coil domains of PML is necessary for its effects on cell survival. Oncogene 16, 2905–2913.
[35] Wang, R. and Brattain, M.G. (2007) The maximal size of protein to diffuse through the nuclear pore is larger than 60 kDa. FEBS Lett. 581, 3164–3170.
[36] Dingwall, C. and Laskey, R.A. (1991) Nuclear targeting sequences – a consensus? Trends Biochem. Sci. 16, 478–481.
[37] Qin, Q., Inatome, R., Hotta, A., Kojima, M., Yamamura, H., Hirai, H., Yoshizawa, T., Tanaka, H., Fukami, K. and Yanagi, S. (2006) A novel GTPase, CRAG, mediates promyelocytic leukemia protein-associated nuclear body formation and degradation of expanded polyglutamine protein. J. Cell Biol. 172, 497–504.
[38] Bohmann, K., Ferreira, J.A. and Lamond, A.I. (1995) Mutational analysis of p80 coilin indicates a functional interaction between coiled bodies and the nucleolus. J. Cell Biol. 131, 817–831.
[39] Sawyer, S.L., Wu, L.I., Emerman, M. and Malik, H.S. (2005) Positive selection of primate TRIM5alpha identifies a critical species-specific retroviral restriction domain. Proc. Natl. Acad. Sci. USA 102, 2832–2837.
[40] Schweiger, S., Foerster, J., Lehmann, T., Suckow, V., Muller, Y.A., Walter, G., Davies, T., Porter, H., van Bokhoven, H., Lunt, P.W., Traub, P. and Ropers, H.H. (1999) The Opitz syndrome gene product, MID1, associates with microtubules. Proc. Natl. Acad. Sci. USA 96, 2794–2799.